Mar Biol (2010) 157:19–27
DOI 10.1007/s00227-009-1292-0

ORIGINAL PAPER

Fatty acid composition as a dietary indicator of the invasive caprellid, *Caprella mutica* (Crustacea: Amphipoda)

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Received: 23 January 2009 / Accepted: 20 August 2009 / Published online: 16 September 2009

Abstract The invasive caprellid amphipod *Caprella mutica* is one of the most widely dispersed marine non-native species globally. Originating in sub-boreal north-east Asia, it has now been found in both the northern and the southern hemispheres. One potential reason why this species is such a successful invader is its ability to utilise a wide variety of food sources. The contribution of different food sources to the diet of *C. mutica* was estimated using fatty acids as biomarkers. *Caprella mutica* was collected from three field sites, including sea cages stocked with Atlantic salmon *Salmo salar*, shellfish longlines stocked with the blue mussel *Mytilus edulis* and mooring lines marking the Loch Linnhe Artificial Reef (>2 km from caged finfish aquaculture), where established populations of this species are known to occur. In addition, the fatty acid compositions of *C. mutica* held in aquaria and either fed the microalga, *Dunaliella tertiolecta*, or the diatom, *Phaeodactylum tricornutum*, for a period of 21 days were investigated. The fatty acid composition of the diatom and the microalgal diets was also examined. The results showed that *C. mutica* contained high levels of polyunsaturated fatty acids, particularly 20:5(n-3); other dominant fatty acids included 18:1(n-9), 22:6(n-3) and 16:0 (in decreasing order based on abundance). Significant differences in the fatty acid profiles between caprellids fed on the microalgae and the diatom diets and between *C. mutica* collected from the field sites were observed. These results provide evidence that lipid biomarkers can be successfully used to provide evidence of feeding strategy for *C. mutica* and that the flexibility observed in this strategy may play an important role in its invasion success.

Communicated by U. Sommer.

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Introduction

The introduction of non-native species is one of the most pervasive, irreversible and devastating impacts of human activity on natural ecosystems. The frequency and distribution of biological invasions is ever-increasing, and it is important to understand the consequences of these invasions on native ecosystems. One of the effects of the introduction of non-native species can be the displacement of native plants and/or animals as a result of competition for food (Porter and Savignano 1990; Byers 2000). Many successful invasive species exhibit a generalist feeding strategy, consuming a wide variety of diet types (Snyder and Evans 2006; Ribeiro et al. 2007; Rocha and Anjos 2007).

Caprella mutica, a non-native epifaunal amphipod, which originates from sub-boreal areas of north-east Asia, was first identified in the United Kingdom on the west coast of Scotland in 2002 (Willis et al. 2004); however, it is now known to have a global distribution (Ashton et al. 2007b; Cook et al. 2007a). This species typically inhabits artificial structures, including aquaculture infrastructure, marina pontoons and boat hulls at extremely high densities (>300,000 m^{-2}) (Ashton 2006) and can dominate sub-littoral

fouling communities at certain times of year (Cook et al. 2006). Yet, little is known about the feeding strategies of this species and the implications that *C. mutica* may have on the abundance of prey items and potential competitors for food in the water column. Stomach content analysis has shown that *C. mutica* is predominantly a detritivore (Guerra-Garcia and Tierno de Figueroa 2009). In addition, it is known that *C. mutica* is capable of filtering particles out of the water column by swaying its body through the water and using its second pair of antennae as a sieve (Nauwelaerts et al. 2007). It is also possible that this species uses a more passive method of particle collection, as other parts of the body of *C. mutica* contain setae which is frequently observed grooming (Nauwelaerts et al. 2007). It has been suggested that caprellid amphipods which have setae on their second antennae obtain a significant part of their diet through filtering and scraping periphyton, whereas caprellids without setae are mainly predators (Caine 1977). *Caprella mutica*, however, is known to consume on average 15 *Artemia* nauplii h^{-1} in laboratory studies (K. Boos, pers. comm.), which suggests that this species can use a variety of different feeding strategies depending on the abundance of potential prey.

The use of fatty acid biomarkers to investigate food web relationships has been used extensively as a reliable method for determining important dietary information over an extended time span (Graeve et al. 1994a, b), unlike stomach content analysis that are both extremely time consuming and can only give an indication of the most recent ingestion of food (Sano et al. 2003). Previous studies have found that diet type can influence the fatty acid composition of crustaceans (Sargent et al. 1987; Falk-Petersen et al. 2000; Virtue et al. 2000), including the Caprellidae (Guerra-Garcia et al. 2004) and that certain fatty acids ("marker lipids"), or their ratios, can be used to provide a more precise indication of an organism's diet and hence it's trophic position. Typical phytoplankton markers are 16:1(n-7), 18:4(n-3) and 18:1(n-7) (Falk-Petersen et al. 2000), 16:1(n-7), C16 PUFA and 20:5(n-3) and a 16:0/16:1(n-7) greater than 1 indicates a dominance of diatoms in the diet (Dunstan et al. 1994; Kharlamenko et al. 1995; Falk-Petersen et al. 2000). Dinoflagellates tend to contain high proportions of 18:4(n-3), 18:5(n-3) and particularly 22:6(n-3) and deficient in 16:1(n-7) (Harrington et al. 1970; Falk-Petersen et al. 2000). The proportion of odd-chained and branched fatty acids and the ratio of 18:1(n-9)/18:1(n-7) fatty acids are also an indicator of bacterial input in the diet (Sargent et al. 1987; Kharlamenko et al. 1995). Carnivorous feeding in marine invertebrates has been associated with 22:6(n-3), 18:1(n-9) and a low ratio 18:1(n-9)/18:1(n-7) (Kharlamenko et al. 1995; Cook et al. 2000). Fatty acids such as 20:1(n-9) and 22:1(n-11) are also thought to be formed by *de novo* biosynthesis in calanoid copepods (Sargent and Whittle 1981; Kattner and Hagen 1995), and the presence of high amounts of these fatty acids reflects animal dietary input (Falk-Petersen et al. 2000). High proportions of 22:1(n-11) are also found in salmon feed pellets, and this fatty acid has been associated with a fish farm-derived diet (Cook et al. 2000).

In a previous study on the fatty acids of *C. mutica* collected from ropes for *Undaria* culture in its native region (Otsuchi Bay, Japan), significant amounts of 20:5(n-3) and 16:1(n-7) and a 16:0/16:1(n-7) ratio of 2.5 (Kawashima et al. 1999) strongly indicate a diatom component to the diet. However, substantial amounts of 18:1(n-9) and relatively high levels of 20:1(n-9) and 22:6(n-3) indicate that this species also had an animal dietary input, including calanoid copepods. Sano et al. (2003) studied the gut contents of *C. mutica* on drifting seaweed off the coast of northern Japan and confirmed that a high proportion of the diet was composed of ephiphytic pennate diatoms and crustaceans. *Caprella mutica* has been successfully reared in aquaria on microalgae and the pennate diatoms (Cook et al. 2007b); however, little is known about the diet of this species in non-native environments.

The aims of this study were to determine the diet and the degree of specificity in the feeding strategy of *C. mutica* in the field using fatty acid biomarkers. Replicate samples were collected from sites where established populations of *C. mutica* are located on the west coast of Scotland including (1) fish farm cage netting, (2) mussel farm longlines and (3) mooring lines for an experimental artificial reef. The fatty acid profiles were determined for these samples and compared to *C. mutica* that either fed (1) the microalga, *Dunaliella tertiolecta*, (2) the pennate diatom, *Phaeodactylum tricornutum* or (3) no additional feed for 21 days in the laboratory. The fatty acid composition of these diets was also obtained, to increase our understanding of the feeding habits of *C. mutica*.

Materials and methods

Sample collection

Caprella mutica was collected from three artificial sites in the Lynne of Lorne, west coast of Scotland in June and July 2005, where established populations are known to occur (Ashton 2006). The sites included sea cages stocked with Atlantic salmon *Salmo salar* (56°27′N, 05°28′W), shellfish longlines stocked with the blue mussel *Mytilus edulis* (56°32′N, 05°20′W) and mooring lines marking the Loch Linnhe Artificial Reef (56°32′N, 05°27′W; > 2 km from caged finfish aquaculture). Treatment groups are hereafter referred to as 'Fish', 'Mussel' and 'Reef', respectively.

Additional *C. mutica* was collected from mooring lines (depth 1–5 m) in the Lynne of Lorne (56°33′N, 05°24′W), west coast of Scotland in August 2005 and maintained in aquaria at the Scottish Association for Marine Science. Plastic mesh (1-mm mesh diameter) provided substratum in the aquaria. Replicate groups of 100 *C. mutica* were held in nine 15-l aquaria for 21 days and fed either (1) the microalga, *D. tertiolecta* (2) the diatom, *P. tricornutum* or (3) given no additional feed, apart from organic material (<5 μm) which would have entered the aquaria via the filtered seawater. Experimental treatment groups are hereafter referred to as 'Algae', 'Diatoms' and 'NF', respectively. Cripps and Atkinson (2000) found that diet could significantly influence fatty acid composition in Antarctic krill, *Euphausia superba* in 16 days, and Cook et al. (2007b) have previously reported that *C. mutica* could be held in aquaria without food for a period of 18 days with approximately 80% survivorship. The microalga, *D. tertiolecta* and the diatom, *P. tricornutum* were supplied by the Culture Collection for Algae and Protozoa (CCAP), Scottish Association for Marine Science (CCAP Collection Code 19/6 B and 1052, respectively). The diatoms were cultured in a semi-continuous culture system (20 l) at 21°C in autoclaved sea water enriched with Walne's medium and a vitamin solution (Walne 1970). The diatom colonies were added ad libitum every 2 days to each container. Any uneaten food and faecal pellets were removed daily. There were 3 replicates of each treatment. The aquaria were aerated, and the sand-filtered seawater was changed every two days prior to feeding. Temperature and photoperiod were maintained at 13 ± 0.5°C and 14 h light/10 h dark to reflect environmental conditions when *C. mutica* are at their peak abundance in the field (Ashton 2006).

Post-experimental treatment or upon collection from the field, male individuals were rinsed with distilled water and stored under nitrogen and frozen prior to analysis. Each of the samples of the diatom and the microalga was filtered onto a GF/F filter and were stored as described for the caprellid tissue.

Tissue preparation and fatty acid analysis

Three replicate samples (each including 10 male caprellids; 20–25-mm body length) from each treatment group and three samples of each dietary source were analysed. Whole male caprellids were used in the analysis as Guerra-Garcia et al. (2004) showed sex differentiation using fatty acid signatures. Analysis of the total lipid fatty acid composition was undertaken as differences between phospholipids and triacylglycerols in amphipods are small (Graeve et al. 2001). Total lipid was extracted from the caprellid material and diatom and microalgal diets by the method developed by Folch et al. (1957). The material was placed in glass boiling tubes, homogenised using a pestle and mortar, and 5 ml of chloroform/methanol (2:1 v/v) containing 0.01% of the antioxidant butylated hydroxyl toluene (BHT) was added. A volume of 50–100 μl 19:0 (1.04 mg/ml) was added to the caprellid and microalga samples, and 50 μl of methyl tricosanoate (23:0) (1.02 mg/ml) was added to the diatom samples to act as an internal standard for gas chromatography (GC) quantification. All samples were stored under nitrogen overnight at 4°C. The supernatant was removed by pipette and passed through a glass wool filter into a boiling tube. Then, 2.5 ml of 0.88% KCl was added to the boiling tube. After shaking and separation of the layers, the layer containing the lipid was removed and evaporated to dryness under nitrogen at 30°C. For analysis of fatty acid composition, the total lipid extracts were subjected to acid-catalysed transesterification. To the lipid extract, 1 ml of toluene and 2 ml of 1% sulphuric acid in methanol were added and left sealed under nitrogen at 100°C for 2 h. The esters were extracted using hexane containing 0.01% BHT and purified using thin-layer chromatography. The fatty acid methyl esters (FAME) extracts were spotted onto silica gel 60 plates and developed with hexane/diethyl ether/glacial acetic acid (80:20:2 v/v) and recovered from the adsorbent with chloroform/methanol (2:1 v/v). FAME extracts were dried under nitrogen, and then 100 μl of hexane (containing 0.015 BHT) was added. Samples were then kept frozen under nitrogen until analysis. FAME were analysed with a Perkin Elmer 8320 gas chromatograph (GC) equipped with a spilt column injector (100:1), flame ionisation detector and a Zebron ZB-WAX-fused silica capillary column (30 m × 0.25 mm i.d.) with helium as the carrier gas. The oven temperature was programmed to rise from 160 to 240°C at 4°C/min and then to hold for 10 min. The detector output was coupled to a computerised data system (Varian Star™) for storage and integration of the chromatograms. Individual components were identified by reference to authentic standards (Sigma, Matreya). Non-saponifiable lipids, which contribute to the total lipid content, however, were not measured in this study as previous studies on related organisms had not recorded that these long chain alcohols and sterols were a major factor in the total lipid content (Kawashima et al. 1999; Graeve et al. 1994a, b; Pond et al. 1997). Individual fatty acids were expressed as the percentage by weight of the total fatty acids characterised.

Statistical analysis

Differences in total fatty acid content and each fatty acid between the different treatment groups (Diatoms, Algae, NF, Fish, Mussel and Reef) were tested using a one-factor ANOVA using the statistical package MINITAB 15. Data were tested for normality prior to analysis using the

Kolmogorov–Smirnov test. Bartlett's test was used to test for homogeneity of variances (Zar 1996) and Tukey's multiple comparison test was used in pair-wise comparisons among treatment groups when significant differences ($P < 0.05$) were determined. Mean results are reported in the text. Multivariate analysis was carried out on the entire data set using the PRIMER v5 (Plymouth Routines In Multivariate Ecological Research; Clarke and Warwick 1994). The data were left untransformed (Howell et al. 2003; Hughes et al. 2006) and converted into similarity matrices using Euclidean distances as the metric. Similarity patterns in the data were visualised using non-metric multidimensional scaling (nMDS). In these two-dimensional plots, individuals with similar fatty acid signatures are placed closer together than those that are more dissimilar. Crossed analysis of similarity (ANOSIM) was performed to examine differences in fatty acid composition between species and different diet types. The similarity percentages procedure (SIMPER) was used to identify the main fatty acids contributing to the similarity measures obtained (Clarke and Warwick 1994).

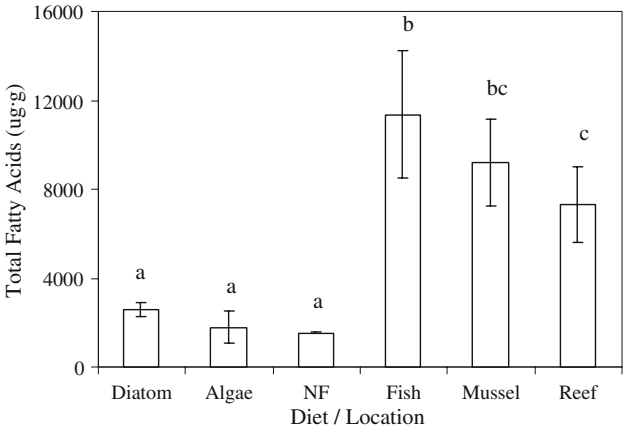

Fig. 1 Total fatty acids (μg g; mean ± SE) in *Caprella mutica* held in aquaria and fed on (1) diatoms, *Phaeodactylum tricornutum* (Diatom), (2) microalga, *Dunaliella tertiolecta* (Algae) and (3) no additional feed (NF) and collected from (1) Atlantic salmon sea cages (Fish), (2) Blue mussel cultivation lines (Mussel) and (3) mooring line at the Loch Linnhe artificial reef (Reef) ($n = 3$). Replicates have been pooled. *Letters* a, b and c indicate statistically significant differences between treatment groups (Tukey test; $P < 0.05$)

Results

Total fatty acid content

A significant difference was found in the total fatty acid content of *C. mutica* between the different treatment groups (ANOVA; df = 5, $F = 15.13$, $P < 0.001$). *Caprella mutica* collected in the field ('Fish', 'Mussel' and 'Reef') were found to contain a significantly higher quantity of fatty acids compared with *C. mutica* held in the aquaria and fed on a monoculture of either diatoms, microalgae or NF (Tukey test, $P < 0.001$) (Fig. 1). *Caprella mutica* collected from 'Fish' also contained higher quantities of total fatty acids compared with samples collected from 'Reef' (Tukey Test, $P < 0.05$) (Fig. 1).

Fatty acid composition

The fatty acid composition of *C. mutica* was characterised by four major fatty acids, including 20:5(n-3), 18:1(n-9), 22:6(n-3) and 16:0 (in decreasing order based on % of total fatty acids) (Tables 1, 2). These comprised between 61.7 and 73.0% of total fatty acids. The fatty acid composition, however, was significantly different between *C. mutica* collected from the three field sites (Fig. 2).

In addition to the major fatty acids found in *C. mutica*, the levels of specific fatty acids, including 16:1(n-7), 18:4(n-3), 20:1(n-9) and 22:1(n-9 + 11), were significantly higher in *C. mutica* collected from 'Fish' compared with 'Reef' ($P < 0.05$). In the case of 22:1(n-9 + 11), this fatty acid in the 'Fish' samples was over double (1.2%) the amount observed in samples from the two other field sites (~0.5%). The majority of these fatty acids were also observed in higher levels at 'Fish' compared with *C. mutica* from 'Mussel', with the exception of 16:1(n-7) ($P < 0.05$). *Caprella mutica* sampled at 'Fish' also had a low 16:0/16:1(n-7) ratio (2.8) (Table 2).

Caprella mutica collected from 'Mussel' had significantly higher levels of 16:1(n-7) and 18:1(n-7) compared with 'Fish' and 'Reef' ($P < 0.05$). 27.3% of the fatty acid composition was 20:5(n-3), which was comparable with the levels of this fatty acid in *C. mutica* from 'Reef', but higher than from 'Fish'. Significantly lower levels of 20:1(n-9), 22:1(n-9) and 22:6(n-3) were also observed in these *C. mutica* compared with the other two field sites ($P < 0.05$). *Caprella mutica* sampled at 'Mussel' had a significantly lower 16:0/16:1(n-7) ratio (2.1) compared with samples from 'Fish' and 'Reef' ($P < 0.05$) (Table 2).

Caprella mutica from 'Reef' had significantly higher levels of 22:6(n-3) (12.67%) compared with 'Fish' and 'Mussel' and higher levels of 20:5(n-3) comparable with samples from 'Mussel'. Significantly lower levels of 16:1(n-7), 18:4(n-3) and 22:1(n-9 + 11) were observed in samples from 'Reef' compared with the other two field sites ($P < 0.05$). *Caprella mutica* from 'Reef' also had a significantly higher 16:0/16:1(n-7) ratio than samples from the other two field sites, but the ratio was still relatively low (3.76) ($P < 0.05$) (Table 2).

Fatty acid composition for *C. mutica* collected from the field sites was significantly different compared with *C. mutica* held in aquaria and fed on known diets (Fig. 2).

Table 1 Fatty acid composition of *Caprella mutica* held in aquarium and fed on (1) diatoms, *Phaeodactylum tricornutum* (Diatom), (2) the microalgae, *Dunaliella tertiolecta* (Algae) and (3) no additional feed (NF) ($n = 3$)

	Caprellids			Diets	
	Laboratory-based				
	Diatom	Algae	NF	*P. tricornutum*	*D. tertiolecta*
14:0	0.37	0.37	0.44	3.34	0.49
15:0	0.22	0.20	0.28	0.20	–
Iso 16.0	0.72	0.76	0.91	–	–
16:0	12.04	14.33	13.98	29.73	16.32
16:1(n-7)	1.92	1.11	0.78	44.50	0.65
16:2	–	1.93	–	0.15	–
17:0	0.83	0.89	0.99	0.05	0.72
17:1	–	–	–	–	2.08
16:3	0.29	0.13	0.26	0.31	–
16:4	–	–	–	0.21	–
DMA 18:0	1.51	1.50	1.73	–	7.22
18:0	2.71	2.72	2.39	0.88	2.16
18:1(n-9)	27.58	20.60	24.77	3.35	16.51
18:1(n-7)	3.68	3.70	2.90	1.97	2.78
18:2(n-6)	2.05	1.87	1.51	0.77	6.01
18:3(n-6)	–	–	–	0.45	–
19:0	–	–	–	–	2.30
18:3(n-3)	0.13	7.29	0.14	0.09	32.71
18:4(n-3)	–	0.33	–	0.13	–
20:0	–	0.11	–	0.00	–
20:1(n-9)	1.78	1.22	2.17	0.02	–
20:1(n-7)	0.24	0.12	0.26	0.02	–
20:2(n-6)	0.54	0.32	0.62	0.11	–
21:0	–	–	–	–	–
20:4(n-6)	4.67	4.63	5.55	1.08	–
20:3(n-3)	0.16	1.49	0.21	0.00	–
20:4(n-3)	0.08	0.17	–	0.07	–
20:5(n-3)	18.67	16.05	18.78	6.60	–
22:1(n-9 + 11)	0.27	0.14	0.35	0.09	–
22:2(n-6)	–	0.22	–	0.29	–
23:0	0.17	0.16	0.07	–	–
22:5(n-3)	0.81	0.30	0.72	1.11	–
24:1	0.32	0.11	0.33	0.22	–
22:6(n-3)	14.76	14.32	15.47	0.62	–
Unident	3.50	2.91	4.37	3.67	10.05
SAFA	17.86	20.28	19.88	34.20	29.21
MUFA	35.78	27.00	31.56	50.17	22.02
PUFA n-3	34.60	39.94	35.32	8.61	32.71
PUFA n-6	7.26	7.05	7.69	2.70	6.01
n-3/n-6	4.76	5.67	4.59	3.19	5.44
18:1(n-9)/18:1(n-7)	7.49	5.57	8.53	1.70	5.94
16:0/16:1(n-7)	6.27	12.86	17.96	0.67	25.11

Fatty acid composition of the diets, *P. tricornutum* and *D. tertiolecta*, are also shown. Replicates have been grouped. The table shows the average percentage of total FAMEs as a % of the total fatty acids

DMA dimethyl acetal, *SAFA* sum of saturated fatty acids, *MUFA* sum of monounsaturated fatty acids, *PUFA* sum of polyunsaturated fatty acids, – not detected

Caprella mutica in the aquaria had significantly higher levels of 18:1(n-9), 20:4(n-6) and 22:6(n-3) and lower levels of 16:1(n-7), 18:4(n-3), 20:5(n-3) and 22:1(n-9 + 11) compared to *C. mutica* collected from the field sites ($P < 0.05$). Within the aquarium-based treatment groups, *C. mutica* fed on the diatom had significantly higher levels

Table 2 Fatty acid composition of *Caprella mutica* collected from (1) Atlantic salmon sea cages (Fish), (2) Blue mussel cultivation lines (Mussel) and (3) mooring line at the Loch Linnhe artificial reef (Reef) ($n = 3$)

	Field sites		
	Fish	Mussel	Reef
14:0	3.66	3.58	2.32
15:0	0.37	0.36	0.26
Iso 16.0	0.56	0.58	0.91
16:0	17.21	15.86	15.88
16:1(n-7)	6.22	7.53	4.23
16:2	0.56	0.88	0.55
17:0	0.37	0.40	0.35
17:1	–	–	–
16:3	0.39	0.35	0.23
16:4	0.31	0.37	0.32
DMA 18:0	1.07	1.56	1.64
18:0	2.07	2.41	2.16
18:1(n-9)	15.24	12.40	14.05
18:1(n-7)	2.72	3.27	3.08
18:2(n-6)	2.19	1.14	0.93
18:3(n-6)	–	–	–
19:0	–	–	–
18:3(n-3)	0.77	0.34	0.21
18:4(n-3)	1.73	0.76	0.52
20:0	–	–	–
20:1(n-9)	2.63	1.39	1.92
20:1(n-7)	0.52	0.67	0.62
20:2(n-6)	0.45	0.33	0.36
21:0	0.20	0.13	0.11
20:4(n-6)	1.77	2.40	2.24
20:3(n-3)	0.26	0.19	0.17
20:4(n-3)	0.64	0.44	0.40
20:5(n-3)	18.57	27.33	27.11
22:1(n-9 + 11)	1.20	0.50	0.45
22:2(n-6)	0.02	0.01	0.01
23:0	0.49	0.42	0.62
22:5(n-3)	1.10	0.99	1.09
24:1	0.34	0.43	0.69
22:6(n-3)	10.72	9.80	12.67
Unident.	5.65	3.18	3.91
SAFA	25.45	24.72	23.35
MUFA	28.87	26.19	25.04
PUFA n-3	33.79	39.85	42.17
PUFA n-6	4.43	3.88	3.54
n-3/n-6	7.63	10.26	11.92
18:1(n-9)/18:1(n-7)	5.61	3.79	4.56
16:0/16:1(n-7)	2.77	2.11	3.76

Fatty acid composition of the diets, *P. tricornutum* and *D. tertiolecta*, are also shown. Replicates have been grouped. The table shows the average percentage of total FAMEs as a % of the total fatty acids

DMA dimethyl acetal, *SAFA* sum of saturated fatty acids, *MUFA* sum of monounsaturated fatty acids, *PUFA* sum of polyunsaturated fatty acids, – not detected

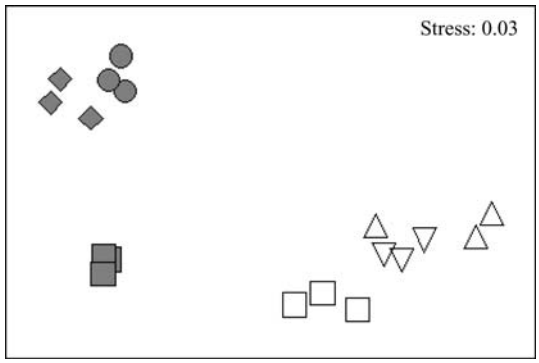

Fig. 2 MDS plot for fatty acid composition for caprellids reared in aquarium on (1) diatoms, *Phaeodactylum tricornutum* (*open triangle*), (2) microalga, *Dunaliella tertiolecta* (*open square*), (3) no additional feed (*open inverted triangle*) and collected from (1) Atlantic salmon sea cages (*filled square*), (2) Blue mussel cultivation lines (*filled diamond*) and (3) mooring line at the Loch Lynne artificial reef site (*filled circle*)

of 16:1(n-7) and a lower 16:0/16:1(n-7) ratio (6.27) than the other two treatment groups reflecting the high quantities of 16:1(n-7) in the diet (44.5%) ($P < 0.05$). *Caprella mutica* that fed on the microalgal diet also contained significantly higher levels of 18:3(n-3) compared with the other treatment groups, again reflecting the high levels of this fatty acid in the diet (32.7%) ($P < 0.05$) (Table 1).

Multivariate analysis of fatty acid signature

Multivariate analysis (ANOSIM) of *C. mutica* showed significant differences in the fatty acid composition between the field sites and aquarium-based treatments (Table 3). No significant difference was found between the aquarium-based treatments ($P > 0.05$). A significant difference was observed between all three field sites (Fig. 2). SIMPER analysis indicated the contribution of certain fatty acids to the average dissimilarity between replicate samples at each sampling site. Four fatty acids contributed most to the differences observed between the three field sites: 16:1 (n-7), 18:1(n-9), 20:5(n-3) and 22:6(n-3) (Table 4).

Discussion

In this study, we analysed the detailed fatty acid compositions of *Caprella mutica* in order to determine the diet and the degree of specificity in the feeding strategy of this species which is non-native to the west coast of Scotland. In the analyses, we used specimens collected from three field sites as well as specimens held in aquaria and fed a specific diet for a period of three weeks. A significant difference was observed between the field and the aquarium-based *C. mutica*, with the latter exhibiting a greater level of particular fatty acids which were found in high abundance in

Table 3 Mutivariate analysis of similarity (ANOSIM) of the fatty acid signature between the replicate samples from each treatment group: diatoms, *Phaeodactylum tricornutum* (Diatom), (2) microalgae, *Dunaliella tertiolecta* (Algae) and (3) no additional feed (NF) and collected from (1) Atlantic salmon sea cages (Fish), (2) Blue mussel cultivation lines (Mussel) and (3) mooring line at the Loch Linnhe artificial reef (Reef)

	R statistic	*p*-Value
Diatom × NF	0.0	>0.05
Diatom × Algae	0.75	>0.05
NF × Algae	1.0	>0.05
Diatom × Fish	1.0	**0.003**
Diatom × Mussel	1.0	**0.006**
Diatom × Reef	1.0	**0.003**
NF × Fish	1.0	**0.015**
NF × Mussel	1.0	**0.022**
NF × Reef	1.0	**0.015**
Algae × Fish	1.0	**0.015**
Algae × Mussel	1.0	**0.022**
Algae × Reef	1.0	**0.015**
Fish × Mussel	1.0	**0.01**
Fish × Reef	1.0	**0.01**
Mussel × Reef	0.865	**0.01**

Significant differences are highlighted in bold

Table 4 Contribution of individual fatty acids to the multivariate differences in the fatty acid signatures between caprellids collected from (1) Atlantic salmon sea cages (Fish), (2) Blue mussel cultivation lines (Mussel) and (3) mooring line at the Loch Linnhe artificial reef (Reef) as determined by the SIMPER (Similarity of Percentages) routine

Fatty acid	Mean (%)		Contribution (%)	Cumulative (%)
	Fish	Mussel		
20:5(n-3)	18.57	27.33	36.14	36.14
18:1(n-9)	15.24	12.40	11.77	47.91
16:1(n-7)	6.22	7.53	6.38	54.29
	Fish	Reef		
20:5(n-3)	18.57	27.11	34.51	34.51
16:1(n-7)	6.22	4.23	8.07	42.58
22:6(n-3)	10.72	12.67	7.88	50.46
	Mussel	Reef		
16:1(n-7)	7.53	4.23	22.9	22.9
22:6(n-3)	9.80	12.67	19.91	42.81
18:1(n-9)	12.40	14.05	13.09	55.90

The mean and SD, percentage contribution (% Cont.) and cumulative percentage (% Cum.), are shown for each fatty acid

the respective diets over a relatively short time period (21 days). These results provide evidence that lipid biomarkers can be successfully used to provide evidence of feeding strategy for this species.

Stomach content analysis of *C. mutica* has previously shown that this species consumes a combination of diatoms and crustaceans in its native region (Sano et al. 2003). The fatty acid analysis of *C. mutica* suggests that this species is adopting a similar omnivorous feeding strategy in the non-native environment, with high levels of 18:1(n-9), 22:6 (n-3) and 20:5(n-3), in all the treatment groups indicating the importance of animal-derived material and diatoms in the diet.

Significant differences in the fatty acid composition were observed between the field sites suggesting that *C. mutica* is able to vary its diet according to available food sources. Elevated levels of 22:1(n-9), a fatty acid that is abundant in both fish feed (Cook et al. 2000) and calanoid copepods (Falk-Petersen et al. 2000), were found in *C. mutica* collected from the Atlantic salmon sea cage site ('Fish') compared with the other field sites ('Mussel' and 'Reef'). Previous work at the same salmon farm found a dominance of the copepods *Pseudocalanus* spp., *Paracalanus* spp. and *Oithona* spp. with densities of ~1000 ind. m^{-3} (Cook, unpubl.). *Oithona* spp. and *Paracalanus* spp. have also been found to dominate plankton samples collected from fish and oyster farm sites in the native region in the Uwa Sea, Japan (Doi et al. 2008). However, the lipid biomarkers were unable to differentiate between fish farm-derived and copepod dietary sources. In addition, high proportions of fatty acids, which indicate the consumption of diatoms, notably 16:1(n-7), a low 16:0/16:1(n-7) ratio, and dinoflagellates 18:4(n-3) were observed in *C. mutica* at the 'Fish' site. Navarro et al. (2008) found increased abundances of phototrophic and heterotrophic plankton in the vicinity of a fish farm on the west coast of Scotland, which may have been an important food source for the caprellids. Again, the biomarkers were unable to determine whether the caprellids were feeding directly on diatoms or on zooplankton which had fed on diatoms. However, stable isotope work by Doi et al. (2008) found that *Caprella* spp. collected from a fish farm in Japan did consume attached microalgae (~60%).

At the mussel cultivation site, significantly higher levels of 20:5(n-3) were found compared with the fish farm site, together with the lowest 16:0/16:1(n-7) ratio, indicating that diatoms formed a greater component of the diet at this site. Doi et al. (2008) also found that *Caprella* spp. at an oyster farm had a greater input of attached microalgae and a reduction in phytoplankton in their diet than at a fish farm at a comparable time of year, potentially reflecting the reduced availability of phytoplankton that is typically observed in the vicinity of shellfish cultivation (Asmus and Asmus 1991; Ogilvie et al. 2000).

At the artificial reef site, however, it was apparent that animal material and/or dinoflagellates play a greater role in the diet of *C. mutica*, with higher levels of 22:6(n-3) (12.67%) compared with the other two field sites. High

levels of 20:5(n-3) also indicate that diatoms are an important dietary component at this site. However, lower levels of the lipid biomarkers for phytoplankton input were observed, which may be attributed to the higher flushing rates experienced at this site compared with the other two field sites (Wilding and Sayer 2002, Cook et al. 2006), which may be attributed to the lack of submerged infrastructure that can significantly reduce water flow (Lo et al. 2007). Populations of *C. mutica* typically expand exponentially during the summer months, reaching a peak in September on the west coast of Scotland (Ashton 2006). The high levels of 22:6(n-3) in the caprellids from the reef site may be attributed to cannibalistic feeding by *C. mutica*, which has been observed in aquaria when held at high densities (Shucksmith 2007). This result could also be explained by the consumption of other amphipods and isopods, which are known to inhabit sub-littoral fouling communities in this region, such as *Jassa* spp. and *Idotea* spp. (Hayward and Ryland 2000, Cook et al. 2006).

In the recipient environment, *C. mutica* has been found to displace the native caprellids *Caprella linearis* and *Pseudoprotella phasma* from substrate (Shucksmith 2007; K. Boos, pers. comm.), potentially competing with these species for similar food types based on the similarity of fatty acid compositions between *C. mutica* in the present study and the native species (Guerra-Garcia et al. 2004). Sano et al. (2003) also observed through stomach content analysis that *C. mutica* had similar proportions of pinnate diatoms and crustaceans as other omnivores, such as *Caprella* spp., *Jassa* spp., *Idotea* spp., and thus, may be in direct competition for food resources in recipient environments. These species are typical members of the sub-littoral fouling communities, and further research is required to determine the extent of competition between *C. mutica* and other ecotrophically similar native fauna.

The aquarium-based *C. mutica* also appeared to have significantly higher levels of 22:6(n-3) compared to the *C. mutica* collected from the field sites, suggesting greater carnivory. Cannibalistic behaviour was observed during the course of the experiment and has been recorded in other laboratory-based studies (Shucksmith 2007; K. Boos, pers. comm.). This may also be linked to an inadequate food supply during the course of the experiment with the caprellids conserving this essential fatty acid, but it does highlight the fact that this species is able to switch between feeding strategies depending on food availability, even exhibiting cannibalistic behaviour when food supply is reduced. *Caprella* spp. have been found in ballast tanks (Carlton 1985) and sea chests (Coutts et al. 2003) in commercial vessels, and its global spread has been attributed to an increase in the frequency and speed of global marine transport (Carlton 2003). The ability of *C. mutica* to survive in such environments during trans-oceanic voyages, however, can be attributed to the flexibility in its feeding behaviour, in addition to the wide physiological tolerances exhibited by this species (Ashton et al. 2007a). The aquarium-based *C. mutica* fed on diatoms, however, did have significantly lower levels of 16:1(n-7) compared with the field-based populations. Previous studies on diet-induced changes in the fatty acid composition of urchin larvae (Liu et al. 2007), adult urchins (Hughes et al. 2006) and arctic herbivorous copepods (Graeve et al. 1994b) have all found that feeding experiments less than 24 days are adequate to see significant changes in the fatty acid composition of the target species. This may indicate that the field populations were able to assimilate greater quantities of a preferred diatom species which was more readily consumed compared with the diatom species used in the aquarium experiment rather than this being an artefact of the length of the feeding period.

In conclusion, fatty acid biomarkers have proved extremely useful in revealing differences between site variation in dietary intake, the omnivorous feeding strategy adopted by *C. mutica* and the flexibility of this species in its ability to assimilate a wide variety of diet types depending on their availability; a trait that can only contribute to the further global spread of this highly successful invasive species.

Acknowledgments We thank the staff of the NERC Scientific Dive Facility with all their help. We also thank Karin Boos for her comments on the manuscript. This research was funded by the Esmée Fairbarin Foundation Marine Aliens Programme, Natural Environment Research Council (NERC) OCEANS 2025, the Scottish Funding Council, Highlands and Islands Enterprise and the European Regional Development Fund, under the Addressing Research Capacity (in the Highlands and Islands) project.

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